ISSN: 0974-5335
IJLST (2014), 7(3):16-21

Comparative Pathological alterations caused by H1N1, H5N1, and H3N2 viruses in human and animal models

Ahmed H. Osman
Professor of Pathology, Faculty of Veterinary Medicine, Cairo University
Ahosman2007@hotmail.com

ABSTRACT
Influenza A viruses are animal disease pathogens that ceaselessly mutated and flow into in many hosts, as birds, pigs, and human. The spectrum of morbidity and mortality of H1N1, H5N1, and H3N2 viruses are related mainly to the pathological lesions they turn out. H1N1 and H5N1 viruses are usually associated with inflammation, congestion and necrosis of the larger airway's epithelium with extension into the alveoli producing interstitial leukocytic infiltration and oedema. High morbidity by H1N1 virus is owned to infection of the epithelium at upper and lower airways. On the other side, seasonal H3N2 virus has primarily displayed inflammation, congestion and tissue injuries of the larger airways with lesser extension into alveoli. Localization of the inflammatory reactions depends upon the presence of virus in membrane tissue cells of the airways, alveolar macrophages, and pneumocytes. Throughout this review, we tend to explain and compare the pathology of these viruses in human cases and animal models.

Key Words: Pathology, Tissue response, receptor specificity and pathogenesis, Influenza A virus, Novel H1N1, avian H5N1, Seasonal H3N2, Human cases, animal models.

INTRODUCTION

Influenza A viruses are characterized by supporting envelope of glycoprotein hemagglutinin (H or HA) and neuraminidase (N or NA). So far, the human population has been confronted on an outbreak scale with three totally different angular distance types: H1, H2, and H3. There's no reason to exclude the likelihood that humans may be infected with all variants, this has already been rumored for H5, H7 and H9 [1]. Most flu virus subtypes are restricted to specific hosts and some appear to be additional promiscuous in many species. Angular distance has a crucial role in deciding host response, because it binds to host cell receptors that contain terminal (α-2,6-SA or α-2,3-SA) [2]. The animal disease origin of human flu pandemics is well described and is highlighted by the increasing range of deadly human infections with H5N1 viruses that have unfolded in domestic birds throughout the components of the East and geographic area, the center East, Africa and Europe [3]. Luckily, these antigenically novel viruses have nevertheless, to sustain human–human transmission and have thus didn't generate a doubtless devastating human pandemic. Among one year, this virus unfolds to 214 countries and caused >18,000 confirmed deaths worldwide [4]. It's calculable that, by Apr 2010, forty three million to eighty nine million folks had been infected with H1N1 2009 [5]. Animals and patients autopsies of infected cases with the various viruses (avian H5N1, swine H1N1, and seasonal H3N2) have disclosed totally different pathologies footage of the respiratory tract beside to morbidity and mortality rates [6]. Pigs are capable of generating resistant flu viruses of pandemic potential, as both the avian and other mammalian influenza viruses can infect pig's epithelial cells in the airways [7].

Pneumonia related to flu is split into primary respiratory disorder viral infection attributable to the virus itself and secondary pneumonia or co-infection with bacteria. Excessive immunological response (cytokine storm) within the host is seen, particularly in primary virus infection [8]. Study of the pathology of confirmed cases is very important to be able to perceive the variations in presenting signs and symptoms, the period of the illness, complications, and mortality that these viruses will turn out. Detailed descriptions of the pathology of cases infected with the pandemic 1918 and 2009 H1N1, H5N1and H3N2 viruses haven't nevertheless appeared within the literature. The descriptions given here are supported by the authors' observations of cases [9].

The pathological lesions encountered mirrors the clinical presentation with the foremost frequent alterations occurred within the airways and lungs.

TISSUE RESPONSE AND RECEPTOR SPECIFICITY:

Even so, the 2009 H1N1 pandemic and former pandemics are reminders that the viruses will promptly bypass host restriction barriers. The angular distance proteins of the human seasonal H1 and H3 virus subtypes chiefly display receptors with terminal α-2,6-SA. In contrast, avian influenza viruses bind preponderantly to brain sugar coupled to α-2,3-SA, that is found extravagantly within the viscera of the birds and lower respiratory tract of humans (10, 11,12) (FIG. 1).

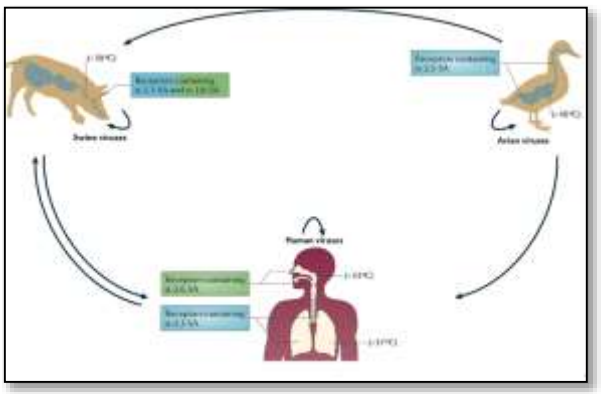

FIG 1: Influenza A virus tropism. The anatomical expression patterns of the viral receptors in different hosts restrict infection with and replication of influenza A viruses. The swine trachea contains receptors with α-2,3-linked and α-2,6-linked sialic acid (α-2,3-SA and α-2,6-SA) moieties that allow for binding of both avian and human viruses, leading to the idea that pigs can serve as the 'mixing vessel.

Pigs have receptors containing each α-2,3-SA and α-2,6-SA in their trachea and have so been projected as a 'mixing vessel' for the reassortment of human and animal viruses, resulting in the potential generation of pandemic viruses. Similarly, pheasants, turkeys, quail and guinea contain each receptor sorts in their intestine [13, 14]. Therefore may additionally function curiously, the H1N1 (SOIV) chargeable for the 2009 pandemic has been recordable to bind to α-2,6-SA and, to a restricted extent, to α-2,3-SA, and may infect cells of the upper and lower respiratory tract [15, 16]. Binding to the lower respiratory tract is believed to induce the pneumonia that's seen in people infected with H1N1 viruses and occurred in some severe cases from the 2009 pandemic [5,17]. However, H5N1 viruses can even infect and replicate in cells of the upper respiratory tract. The specificity of avian viruses for α-2,3-SA containing receptors, that in humans are chiefly gift within the lower respiratory tract, most likely contributes to the restricted avian–human infectious agent transmission.

Pandemic H1N1 strain would associate with increase of replication rate, transmission and pathological process that observed in animal models for this virus compared with seasonal H3N2 viruses. The severity of the illness evoked by the 2009 pandemic H1N1 virus within the general human population wasn't drastically completely different to it seen with seasonal respiratory disease, suggesting that extra factors, like pre-existing immunity and host diversification, modulate the infective potential of respiratory disease A viruses in humans [18].

PATHOLOGY OF INFLUENZA A VIRUSES

PATHOLOGY OF H1N1

The pathology of deceased patient's lung infected with the pandemic 1918 and 2009 H1N1 viruses have shown primarily diffuse alveolar damage and collapse with extensive hyaline membrane formation and interstitial edema (Figure 2A). Thrombosis of alveolar blood vessels, which has led to massive necrosis of the alveolar walls (Figure 2B) and varying degrees of mixed inflammatory cells infiltration mainly neutrophils, macrophages, plasma cells and lymphocytes. Alveolar epithelial cells and pneumocytes showed reactive changes, and their nuclei seem vesiculated. Alveolar spaces revealed desquamated pneumocytes, blood and erythrophagocytosis. Lungs showed proliferation of fibroblasts in patients infected with low virulence viruses. Immunohistochemical assays have demonstrated viral antigens in pneumocytes type I and II and intra-alveolar macrophages [19].

Bacterial pneumonia, showed intra-alveoli accumulation of neutrophils [20, 21]. The trachea and major bronchi of deceased patients infected with the pandemic 1918 and 2009 H1N1 revealed necrosis and desquamation of the mucosal epithelium with oedema and mixed inflammatory infiltrate the submucosa (Figure 2C). The mucous glands in these airways showed sloughing of goblet cells and necrosis of surrounding submucosa (Figure 2D). Viral antigens noted in the epithelial cells and mucous glands of trachea, bronchi, and bronchioles as that recorded by (Centers for Disease Control and Prevention, Atlanta, GA, 2009). Myocardial infarction was documented in several patients infected with the 1918 and 2009 H1N1 viruses [20, 21].

Several animal models used to study the pathology of H1N1 infections and the results vary depending on the host animal susceptibility and virulence of virus used for the infection [21, 22, and 23]. In guinea pigs and pigs, low pathogenic seasonal H1N1 virus (A/Puerto Rico/8/34 and A/swine/Thailand/HF6/05) showed viral antigens in the mucosal epithelium of the upper airways and bronchioles. Damage of the mucosal epithelium associated with hyper-production of mucous and mild inflammatory infiltrate. Per-bronchial inflammatory reaction consisted of congestion, oedema and inflammatory cells infiltration [24]. In ferrets, Low pathogenic seasonal H1N1 showed multifocal necrotizing rhinitis tracheitis, bronchitis, and bronchiolitis with abundant viral antigens in the nasal turbinate's and focal consolidation of the lungs without evidence of viral antigens in the lower respiratory tract [25]. Influenza H1N1 viruses replicated to higher titers in lung tissue and can be recovered from the upper and lower airways as well as the intestinal tract [15]. In mice, Low pathogenic seasonal H1N1 virus, lungs showed diffuse consolidation accompanied with bronchitis and interstitial pneumonia. The thymus showed depletion of cortical lymphocytes. Necrotic debris was noted in the lumen of bronchioles and alveoli. Viral antigens were localized in bronchiolar epithelium and alveolar macrophages. Focal

aggregation of lymphocytes, macrophages and plasma cells were seen in cardiac muscle [25, 26 and [27].

PATHOLOGY OF H5N1

The pathology of deceased patient's lung infected with the H5N1 virus has shown diffuse alveolar damage and hyaline membrane formation. The alveolar interstitium showed oedema and congestion of capillaries with leukocyte infiltration mainly lymphocytes and macrophages. Fibroblast proliferation also detected at (5 to 9 days post-infection) (Figure 3A) [28, 29]. Intra-alveolar hemorrhage, hyperplasia of type II pneumocytes and hemophagocytosis noted not only in the lung but also in bone marrow, lymph nodes (Figure 3B&C), spleen, and liver. Other organs were also affected including, spleen with depletion of white pulp. Kidneys showed acute tubular necrosis especially the proximal convoluted tubules. On the other side, liver of infected patient revealed fatty change and coagulative necrosis of hepatocytes beside to hyperactivity of Kupffer cells, and cholestasis. Focal demyelinated areas in the brain with necrosis and reactive histiocytes seen in some patients [30, 31, and 32].

The animal models that used include mice, ferrets, and pigs. Macroscopically, all animals showed various degrees of consolidation and discoloration in the lungs and hemorrhages in adipose tissue surrounding liver, intestines, kidneys, and bladder [33, 34]. Macroscopic changes occurred earlier in animals infected with the highly virulent viruses in comparison to those infected with low-virulence viruses. Microscopically, the lungs showed intra-alveolar hemorrhage, leukocytes and desquamated cells beside to edema surrounding bronchioles which extend into the alveolar septae [33, 30]. At about 3 days post-inoculation viral antigens detected in type II pneumocytes [3]. The brains of infected animals with the highly virulent viruses showed glial nodules, perivascular cuffing by lymphocytes and neuronophagia, [3, 33]. Viral antigens have been also detected in neurons.

PATHOLOGY OF H3N2

The pathological changes seen in deceased patients with acute respiratory infection showed congestion of the trachea, major bronchi and bronchioles and sub-mucosal hemorrhage (Figure 4A & 4B) [34].

Uneven protein alveolar exudates, hyaline membrane formation, oedema, and proliferation of pneumocytes were seen [23,34and35]. On the opposite side, chronic respiratory injury characterized microscopically by inflammatory infiltrates primarily mononuclear cells and organization of air spaces and interstitium tissues [34, 35].

Viral antigens could solely be gift within the cartilaginous tube of the respiratory tract. In other side, the cardiac muscle showed patchy areas of necrosis infiltrated with mononuclear cells (Figure 4C).

There are many descriptions of animals infected with the H3N2 virus that have typically been compared to animals that are infected with seasonal H1N1 viruses as mice, guinea pigs, pigs and ferrets [5, 8, 10]. Altogether of those animal models lungs showed consolidation. Microscopically there's shedding and disorganization of the epithelium, congestion and leukocytic infiltration of the airways membrane together with (nasal, paranasal, sinuses, trachea, bronchus, and bronchioles) beginning at one day post-infection. Changes in the respiratory mucosa are accompanied by decline sharply of viral antigens within 3 days post-infection. Viral antigens detected within alveolar macrophages. Extra-pulmonary, infectious agent antigens are found within the heart and thymus. In pigs, lesions produced by seasonal H1N1 were more extensive than those produced by infections with the H3N2 virus (10).

Pathological changes in organs aside from the lungs were primarily secondary to multiple organ failure. Though, there have been no histologic signs of extra-pulmonary virus–induced sickness, we tend to don't gift any knowledge on the presence of infectious agent RNA/protein in alternative organs. However, sound proof for replication of respiratory disease virus in extra-respiratory tissues remains missing (30).

PATHOGENESIS OF INFLUENZA A VIRUSES

The pathogenesis of severe lung injury related to influenza A viruses infection in humans is poorly understood. The antiviral mechanisms may be related to $CD8^+$ T cells that led to direct lysis of infected cells or production of inflammatory cytokines. However, $CD8^+$ T cells also contribute to tissue injury in the course of a viral infection. Immunopathology caused by $CD8^+$ T cells is clearer in cases of high viral dose, when the T-cell response does not control viral loads [35].

An influenza virus–induced “cytokine storm” is believed to be involved in the pathogenesis of severe forms of influenza [36]. The high circulating levels of cytokines, such as IFN-γ and TNF-α, associated with the erythrophagocytosis observed in severe infections. In experimental models of influenza infection, the role of TNF-α and, to a lesser extent, of IFN-γ in lung immunopathology was recorded [37]. The pathology caused by these viruses in humans or animal models depends on the virulence of the infecting agent and host response. All the viruses infect the respiratory epithelium through the nasal passages to bronchioles;

however, high virulence viruses (1918 H1N1, H5N1, and probably 2009 H1N1) also tend to infect pneumocytes and intra-alveolar macrophages. Thus, low-virulence viruses (seasonal H3N2 and H1N1) cause primarily congestion, leukocytic infiltration and epithelial sloughing of the larger airways (trachea, bronchi and bronchioles). On the other side, high-virulence viruses revealed diffuse alveolar damage [15, 16]. In addition, the pathological picture will change to intra-alveolar neutrophilic infiltrate if bacterial super-infection is present. The use of antibiotics, supportive measures (ventilators), and vaccinations for *S. pneumoniae and Haemophilus influenza* will probably cut the frequency of these super-infections but will bring community and nosocomial bacterial infections that will have acquired antibiotic resistance [22].

However, three distinct patterns of lung lesions among infected patients were clinical relevance. Patients with necrotizing bronchiolitis had a more severe neutrophil-predominant inflammatory exudate compared with the others. Previous reports on the pathology of influenza A viruses co-infected by bacteria indicated by presence of many neutrophils in the lung tissue strongly suggests a bacterial co-infection [28]. Furthermore, in the group of patients with severe alveolar hemorrhage, no alveolar cell viral cytopathic changes could be detected. These findings suggest that severe alveolar hemorrhage considered as co-morbidities, such as chronic cardiovascular disease and coagulopathies, conditions that predispose the patients to increased alveolar pressure and bleeding [10].

CONCLUSIONS

The complicated dynamics of influenza A viruses unendingly challenges host species barriers, thus the novel virulent virus strains in humans is a relentless risk. To make achieve insights into the mechanism by that reassorted viruses arise, any studies area unit necessary to explain the particular receptor necessities and also the specific cell sort and organ wherever such an incident may happen. Moreover, during this advanced interaction between host and influenza A viruses, host factors play a very important role in the severity and outcome, and studies that specialize in distinctive the human susceptibleness factors area units. Therefore, the key for understanding the host determinants of respiratory disorder virus is pathologic process. Current animal model systems solely part sum up human respiratory disorder, therefore complementary, fastidiously designed clinical studies area unit required to confirm such models and to seek out the genetic susceptibleness factors and polymorphisms that modulate disease outcome in humans.

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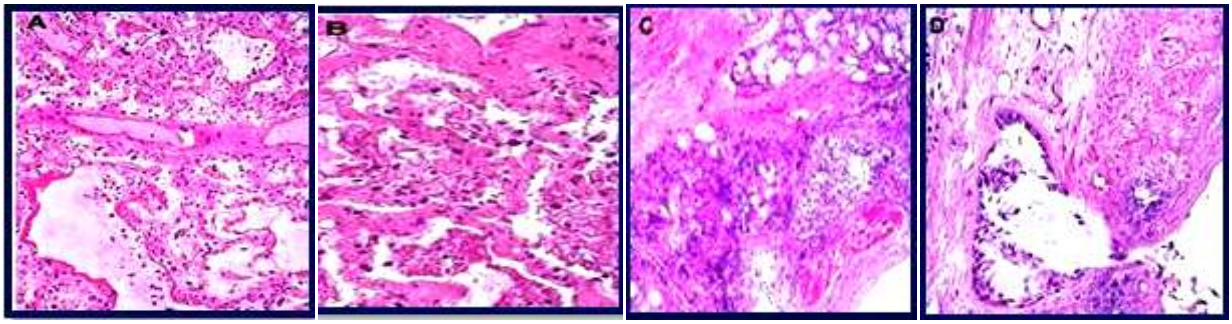

Figure 2. Histopathological characteristics of fatal cases with confirmed pandemic 2009 H1N1 virus infection showing diffuse alveolar damage in the early exudative phase characterized by hyaline membranes (A,B), intraalveolar edema (A), and alveolar wall necrosis (B) accompanied by inflammatory infiltrate (A). The trachea and bronchi showed denuded mucosa (C,D) with inflammation (C) and necrosis (D) of the submucosa. H&E stains; original magnifications: X10 (A), X20 (B,D), and X5 (C). **Archives of Medical Research 40 (2009) 655e661**

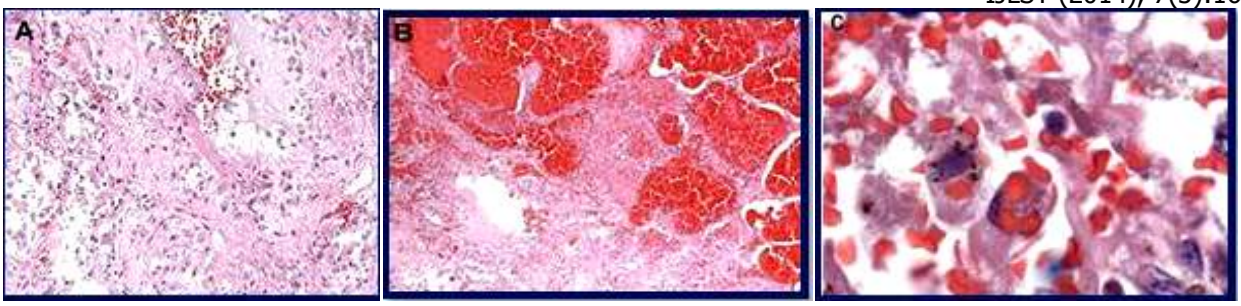

Figure 3. Histopathological characteristics of fatal cases with confirmed H5N1 virus infection showing diffuse alveolar damage in the later fibrous proliferative phase characterized by fibroblast proliferation (A) and deposition of collagen (A,B). Intra-alveolar hemorrhage is seen in some cases (B) as well as hemophagocytosis (C). H&E stains; original magnifications: X10 (A), X5 (B), and X63 (C). **Archives of Medical Research 40 (2009) 655e661**

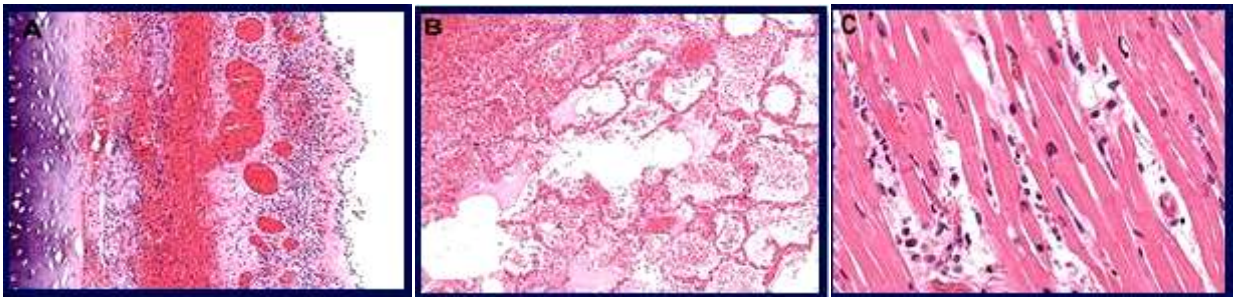

Figure 4. Histopathological characteristics of fatal cases with confirmed seasonal H3N2 virus infection showing mucosal desquamation and disorganization and submucosal congestion, hemorrhage, and inflammation in the trachea (A). The lungs show intra-alveolar hemorrhage and edema (B). The heart shows focal areas of mononuclear inflammatory infiltrate (C). H&E stains; original magnifications: x10 (A), x5 (B), and x40 (C). **Archives of Medical Research 40 (2009) 655e661**